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APPLICATION NUMBER: 60/453,771

FILING DATE: *March 11, 2003*

RELATED PCT APPLICATION NUMBER: *PCT/US03/33578*



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AIP Rev

03/11/03
J1064 U.S. PTO

Attorney's Docket No. 9345.2PR2

PATENTJ1036 U.S. PTO
60/453771
03/11/03**COVER SHEET FOR FILING PROVISIONAL
PATENT APPLICATION (37 CFR §1.51(c)(1))**Box PROVISIONAL PATENT APPLICATION
Commissioner for Patents
Washington, DC 20231

March 11, 2003

This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 C.F.R.
§1.53(c).

Docket No.	9345.2PR2
Type a plus sign (+) inside this box <input type="checkbox"/>	+

INVENTOR(s)/APPLICANT(s)Name: Mao-Hsiung Yen
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Address:TITLE OF THE INVENTION (280 characters maximum)
BAICALEIN AND BAICALEIN ANALOGS AND USES THEREOFENCLOSED APPLICATION PARTS (check all that apply)

- ☒ Specification (Number of Pages 40)
- ☐ Drawing(s) (Number of Sheets)
- ☒ Claims (Number of Claims 92)
(A complete provisional application does not require claims 37 C.F.R. §1.51(c)).
- ☐ Application Data Sheet. See 37 CFR §1.76
- ☐ Other:

In re: Yen, et al.
Filed: Concurrently Herewith
Page 2

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Respectfully submitted,

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Vickie Diane Prior

Attorney Docket No. 9345-2PR2

BAICALEIN AND BAICALEIN ANALOGS AND USES THEREOF

Mao-Hsiung Yen and Yuan-Sheng Wu

Field of the Invention

The present invention concerns flavonoids, novel flavonoid analogs, and pharmaceutical formulations thereof, and use thereof for prevention and treatment of disorders such as septic shock.

Background of the Invention

Septic shock can be defined as a spectrum of clinical conditions caused by the immune response of a host to infection or trauma characterized by systemic inflammation and coagulation (Mesters RM, et al. Increase of plasminogen activator inhibitor levels predicts outcome of leukocytopenic patients and sepsis. *Thromb Haemost.* 1996a; 75: 902-907; Wheeler AP and Bernard GR. Treating patients with severe sepsis. *N Engl J Med.* 340: 207-214 (1999)). Conditions range from a systemic inflammatory response to organ dysfunction to multiple organ failure, and ultimately death. In elderly, immunocompromised, and critically ill patients, septic shock is a major cause of morbidity and mortality in intensive care units worldwide (Friedman G, et al. Has the mortality of septic shock changed with time? *Crit Care Med.* 26:2078-2086 (1998)). In the United States, septic shock is the leading cause of death in noncoronary intensive care unit (ICU) patients (Sands KE, et al. Epidemiology of sepsis syndrome in 8 academic medical centers. *JAMA* 278: 234-240 (1997)). Additionally, 1998 data from the Centers for Disease Control show that septic shock is the 11th leading cause of death overall (National Vital Statistics Report, 2000).

Flavonoids or bioflavonoids encompass a ubiquitous group of polyphenolic substances that are present in most plants, concentrated in seeds, fruit skin or peel, bark, and flowers. Various classes of flavonoids include the following: flavanols, flavanones, flavones, flavan-3-ols (catechins), anthocyanins, and isoflavones. Baicalein, baicalin, and wogonin (shown below) are known bioactive flavonoids of

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Scutellaria baicalensis GEORGI. In recent studies, baicalein, baicalin, and wogonin have been reported to show anti-inflammatory (Bao, QL et al. (2000) The flavonoid baicalin exhibits anti-inflammatory activity by binding to chemokines. *Immunopharmacology*. 49: 295-306; Wakabayashi I and Yasui K (2000) Wogonin inhibits inducible prostaglandin E₂ production in macrophages. *Eur J Pharmacol*. 406: 477-481; Kimura et al. (1997) Effects of baicalein isolated from *Scutellaria baicalensis* on interleukin 1 β - and tumor necrosis factor α -induced adhesion molecule expression in cultured human umbilical vein endothelial cells. *J Ethnopharmacol*. 57: 63-67; and Lin CC and Shieh DE (1996) The anti-inflammatory activity of *Scutellaria rivularis* extracts and its active components, baicalin, baicalein and wogonin. *Am J Chin Med*. 24: 31-36), anti-allergic (Kyo et al. (1998) Baicalin and baicalein, constituents of an important medicinal plant, inhibit intracellular Ca²⁺ elevation by reducing phospholipase C activity in C6 rat glioma cells. *J. Pharm Pharmacol*. 50: 1179-1182; Gao et al. (1999) Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of *Scutellaria baicalensis* Georgi. *Biochemica et Biophysica Acta B*. 1472: 643-650; Gabrielska J (1997) Antioxidant activity of flavones from *Scutellaria baicalensis* in lecithin liposomes. *J Biosci*. 52: 817-823), antioxidant (Shieh et al. (2000) Antioxidant and free radical scavenging effects of baicalein, baicalin, and wogonin. *Anticancer Res*. 20: 2861-2865), and anticancer activities (Ikemoto S et al. (2000) Antitumor effects of *Scutellariae Radix* and its components baicalein, baicalin, and wogonin on bladder cancer cell lines. *Urology* 55: 951-955; Chan FL et al. (2000) Induction of apoptosis in prostate cancer cell lines by flavonoid, baicalin. *Cancer Lett.*). Moreover, baicalin has been shown to possess antiviral activity (Nagai T et al. (1995) Mode of action of the anti-influenza virus activity of plant flavonoid, 5,7, 4'-trihydroxy-8-methoxyflavone, from the roots of *Scutellaria baicalensis*. *Antiviral Res*. 26: 11-25; Nagai T et al. (1995) Mode of action of the anti-influenza virus activity of plant flavonoid, 5,7,4'-trihydroxy-8-methoxyflavone, from the roots of *Scutellaria baicalensis* and enhancement of its activity by drug delivery system. *Antiviral Res*. 30: A1-A62; and Kitamura K et al. (1998) Baicalin, an inhibitor of HIV-1 production *in vitro*. *Antiviral Res*. 37: 131-140), and baicalein has been shown to produce a hypotensive effect (Takizawa et al.

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(1998) Prostaglandin I₂ contributes to the vasodepressor effect of baicalein in hypertensive rats. *Hypertension* 31: 866-871 and Chen ZY et al. (1999) Endothelium-dependent contraction and direct relaxation induced by baicalein in rat mesenteric artery. *Eur J Pharmacol*, 374:41-47). More recently, baicalein has been implicated in the inhibition of expression of adhesion molecules induced by cytokines in human umbilical vein endothelial cells (Ikemoto, S et al. Antitumor effects of *Scutellariae radix* and its components baicalein, baicalin, and wogonin on bladder cancer cell lines. *Urology*. 55: 951-955 (2002); Middleton, EJ and Kandaswani C. Effects of flavonoids on immune and inflammatory cell functions. *Biochem. Pharmacol.* 43: 1167-1179 (1992). However, the use of baicalein and analogs thereof, baicalin, and wogonin in the prophylaxis and treatment of septic shock has not been reported in the literature.

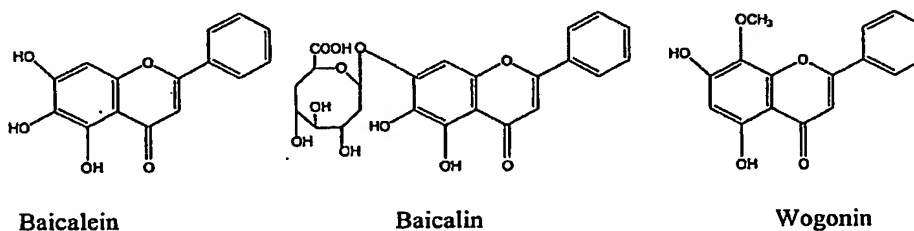


Chart 1. Bioactive flavonoids of *Scutellaria baicalensis* GEORGI.

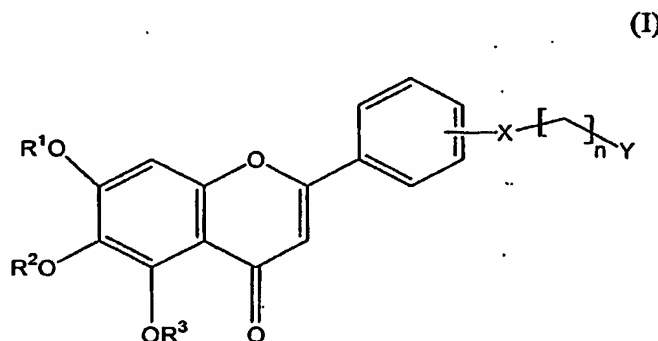
Current therapies for the treatment of septic shock include antibiotics, vasoconstrictors, steroids, and fluid supplementation to maintain the circulation volume; however, in many cases, these therapies have been deemed inefficient (Barron RL. Pathophysiology of septic shock and implications for therapy. *Clin. Pharm.* 12: 829-845 (1993)). It is desirable to provide compounds useful for the prevention or treatment of septic shock.

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Summary of the Invention

When compared to conventional therapies described above, the present invention may provide a more useful therapy for the prevention or treatment of septic shock.

According to embodiments of the present invention, the present invention relates to a compound according to formula I:



wherein:

R^1 , R^2 , and R^3 are each independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, and carbohydrates;

or R^1 and R^2 together are heterocycles;

or R^2 and R^3 together are heterocycles;

X is selected from the group consisting of H, C, N, NR' , $NR'R''$, $NR'SO_2R''$, O, and S, subject to the proviso that the compound according to formula I is not baicalein, and wherein R' and R'' are each independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, and carbohydrates; and wherein when X is $NR'R''$ or $NR'SO_2R''$, n is 0 and Y is not present, and R' and R'' together form a 5 to 7-membered ring;

n is from 0 to 3; and

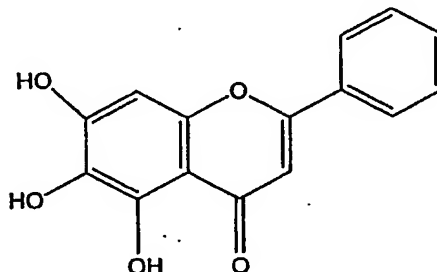
Y is selected from the group consisting of OR^4 and NR^5R^6 , wherein R^4 , R^5 , and R^6 are each independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, and carbohydrates, and R^5 and R^6 together may form a 5 to 7-membered ring; or pharmaceutically acceptable salts thereof.

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According to other embodiments of the present invention, the invention relates to method of preventing or treating conditions selected from the group consisting of septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders, comprising administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of a compound of formula I described above.

According to still other embodiments, the present invention relates to a method of preventing or treating conditions selected from the group consisting of septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders, comprising administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of a compound of the formula II:

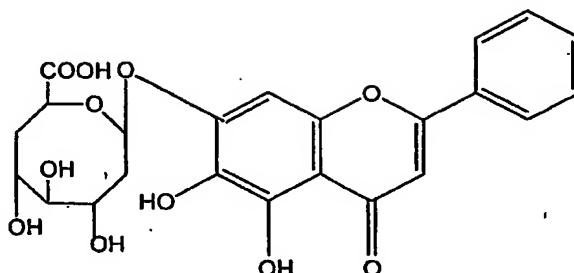
(II)



According to yet other embodiments, the present invention relates to a method of preventing or treating conditions selected from the group consisting of septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders, comprising administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of a compound of the formula III:

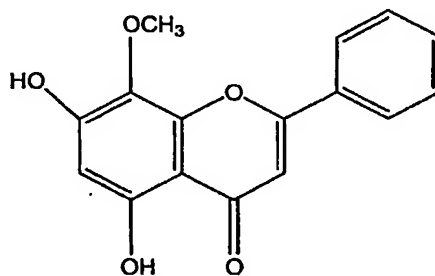
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(III)



According to still other embodiment, the present invention relates to a method of preventing or treating conditions selected from the group consisting of septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders, comprising administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of a compound of the formula IV:

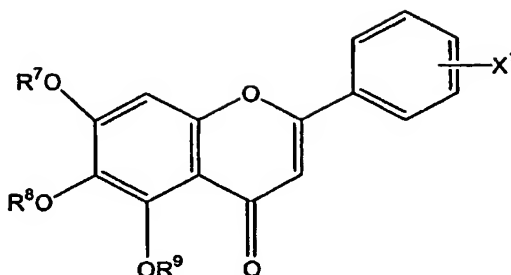
(IV)



According to yet other embodiments of the present invention, the present invention relates to a method of preventing or treating conditions selected from the group consisting of septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders, comprising administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of a compound of the formula V:

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(V)



wherein:

R^7 , R^8 , and R^9 are each independently selected from the group consisting of H, alkyl, carbohydrates, and benzyl;

or R^7 and R^8 together are heterocycles;

or R^8 and R^9 together are heterocycles;

X^1 is selected from the group consisting of H, C, NH_2 , $NHCOCH_3$, NO_2 , and OR^{10} , wherein R^{10} is selected from the group consisting of H, alkyl, carbohydrates, and benzyl; or pharmaceutically acceptable salts thereof.

According to further embodiments, moieties can be selected to confer water solubility to the compound(s).

Brief Description of Drawings

Figure 1 illustrates syntheses of the compounds of the present invention.

Figure 2 illustrates effects of baicalein on mean arterial blood pressure in rats treated with endotoxin.

Figure 3 illustrates effects of baicalein on the change of mean arterial blood pressure in rats treated with endotoxin.

Figure 4 illustrates effects of baicalein on the change of heart rate in rats treated with endotoxin.

Figure 5 illustrates effects of baicalein treatment on the concentration-response curve of norepinephrine in aortic rings from lipopolysaccharide-treated rats.

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Figure 6 illustrates effects of baicalein treatment on the concentration-response curve of acetylcholine in aortic rings from lipopolysaccharide-treated rats.

Figure 7 illustrates effects of baicalein treatment on the concentration-response curve of L-arginine in aortic rings from lipopolysaccharide-treated rats.

Figure 8 illustrates effects of baicalein treatment on the plasma TNF- α level in lipopolysaccharide-treated rats.

Figure 9 illustrates effects of baicalein on superoxide anion in rats treated with endotoxin.

Figure 10 illustrates effects of baicalein on inducible nitric oxide synase (iNOS) protein expression in rats treated with endotoxin.

Figure 11 illustrates effects of baicalein on I κ B α protein degradation in rats treated with endotoxin.

Figure 12 illustrates effects of baicalein on the survival rate of mice treated with endotoxin.

Detailed Description of the Invention

The present invention will now be described more fully hereinafter with reference to the accompanying figures, which further illustrate the invention described herein. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the invention and the appended claims, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

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All publications, patent applications, patents and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

The term "alkyl" as used herein refers to C1-C20 inclusive, linear, branched, or cyclic, saturated or unsaturated hydrocarbon chains, including for example, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl, octyl, ethenyl, propenyl, butenyl, pentyl, hexenyl, octenyl, butadienyl, and allenyl groups. Alkyl groups can either be unsubstituted or substituted with one or more non-interfering substituents, e.g., halogen, alkoxy, acyloxy, hydroxy, mercapto, carboxy, benzyloxy, phenyl, benzyl, or other functionality which has been suitably blocked with a protecting group so as to render the functionality non-interfering. Each substituent may be optionally substituted with additional non-interfering substituents. The term "non-interfering" characterizes the substituents as not adversely affecting any reactions to be performed in accordance with the process of this invention.

"Loweralkyl" as used herein refers to C1 to C4, C6 or C8 alkyl, which may be linear or branched and saturated or unsaturated.

"Cycloalkyl" is specified as such herein, and is typically C3, C4 or C5 to C6 or C8 cycloalkyl.

The term "hydroxyalkyl" as used herein refers to C1 to C4 linear or branched hydroxy-substituted alkyl, i.e., $-\text{CH}_2\text{OH}$, $-(\text{CH}_2)_2\text{OH}$, etc.

The term "aminoalkyl" as used herein refers to C1 to C4 linear or branched amino-substituted alkyl, wherein the term "amino" refers to the group $\text{NR}'\text{R}''$, wherein R' and R'' are independently selected from H or lower alkyl as defined above, i.e., $-\text{NH}_2$, $-\text{NHCH}_3$, $-\text{N}(\text{CH}_3)_2$, etc.

The term "oxyalkyl" as used herein refers to C1 to C4 oxygen-substituted alkyl, i.e., $-\text{OCH}_3$, and the term "oxyaryl" as used herein refers to C3 to C10 oxygen-substituted cyclic aromatic groups.

The term "alkenyl" refers to a hydrocarbon group, typically C2 to C4, derived from the corresponding alkyl and which contains at least one double bond (e.g., butadienyl). "Loweralkenyl" as used herein likewise refers to C1 to C4 alkenyl.

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"Alkynyl" refers to a hydrocarbon group, typically C2 to C4, derived from the corresponding alkyl and which contains at least one triple bond (*e.g.*, butadiynyl).

The term "aryl" as used herein refers to C6 to C10 cyclic aromatic groups such as phenyl, naphthyl, and the like, and includes substituted aryl groups such as tolyl.

"Heterocycle" as used herein refers to a monovalent saturated, unsaturated, or aromatic carbocyclic group having a single ring or multiple condensed ring and having at least one hetero atom, such as N, O, or S, within the ring, which can optionally be unsubstituted or substituted with hydroxy, alkyl, alkoxy, halo, mercapto, and other non-interfering substituents. Examples of nitrogen heterocycles include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, and indoline.

"Halo" as used herein refers to any halogen group, such as chloro, fluoro, bromo, or iodo.

The term "carbohydrate" as used herein refers to a compound which is either a carbohydrate per se made up of one or more monosaccharide units having at least 6 carbon atoms (which may be linear, branched or cyclic) with an oxygen atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which may be linear, branched or cyclic), with an oxygen atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri-, and oligosaccharides), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C6 and above (preferably C6 to C8) sugars such as glucose, fructose, mannose, galactose, ribose, and sedoheptulose; di- and trisaccharides would include sugars having two or three monosaccharide units (preferably C5 to C8) such as sucrose, cellobiose, maltose, lactose, and raffinose.

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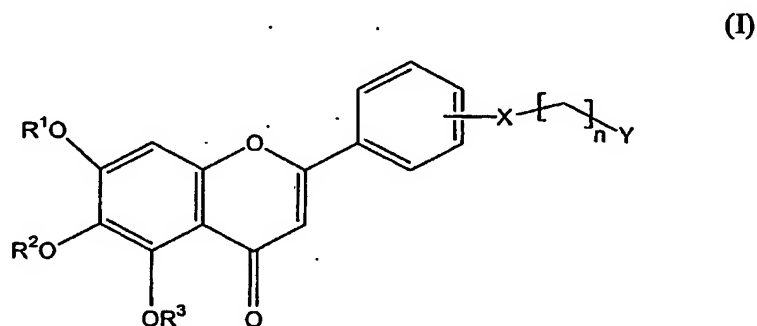
The terms "flavonoids" or "bioflavonoids" as used herein relate to a ubiquitous group of polyphenolic substances which are present in most plants, concentrated in seeds, fruit skin or peel, bark, and flowers. Various classes of flavonoids include the following: flavanols, flavanones, flavones, flavan-3-ols (catechins), anthocyanins, and isoflavones. Exemplary flavonoids include, but are not limited to, baicalein, baicalin, wogonin, and analogs thereof.

"Treat" or "treating" as used herein refers to any type of treatment that imparts a benefit to a patient afflicted with a disease, including improvement in the condition of the patient (e.g., in one or more symptoms), delay in the progression of the condition, prevention or delay of the onset of the disease, etc.

As used herein, a "pharmaceutically acceptable" component (such as a salt, carrier, excipient or diluent) means that the compound or composition is suitable for administration to a subject to achieve the treatments described herein, without unduly deleterious side effects in light of the severity of the disease and necessity of the treatment.

"Therapeutically effective amount" as used herein refers to an amount necessary to prevent, delay or reduce the severity of the condition of interest and also includes an amount necessary to enhance normal physiological functioning.

In general, active compounds of the present invention comprise a structure according to the following formula:



wherein:

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R^1 , R^2 , and R^3 are each independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, and carbohydrates;

or R^1 and R^2 together are heterocycles;

or R^2 and R^3 together are heterocycles;

X is selected from the group consisting of H, C, N, NR' , $NR'R''$, $NR'SO_2 R''$, O, and S, wherein R' and R'' are each independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, and carbohydrates; and wherein when X is $NR'R''$ or $NR'SO_2 R''$, n is 0 and Y is not present, and R' and R'' together form a 5 to 7-membered ring;

n is from 0 to 3; and

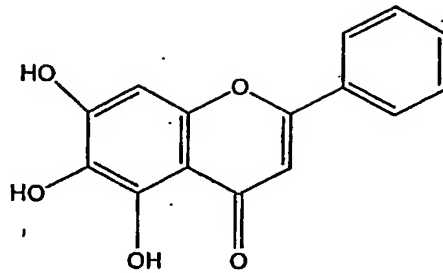
Y is selected from the group consisting of OR^4 and NR^5R^6 , wherein R^4 , R^5 , and R^6 are each independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, and carbohydrates, and R^5 and R^6 together may form a 5 to 7-membered ring; or pharmaceutically acceptable salts thereof, and X may be attached in either an ortho, meta, or para relationship.

The compound according to formula (I) can be water soluble. In some embodiments, R^1 , R^2 , and R^3 can be a moiety selected to confer water solubility to the compound to provide a novel, water-soluble compound. In addition to the description above, R^1 , R^2 , and R^3 can be a sulphate, sulphonate, phosphate, or phosphonate as selected to provide a novel, water-soluble compound. Moreover, the sulphate, sulphonate, phosphate, or phosphonate can be in the form of a water-soluble salt. As a representative non-limiting example, a water-soluble salt can be formed using an alkali metal salt, such as sodium, potassium, or ammonium. The water-soluble salt may be in the form of a mono, di-, or tri- alkali metal salt. In some embodiments, R^1 , R^2 , and R^3 can be SO_3H or PO_3H_2 and salts thereof, including, but not limited to, sodium and potassium.

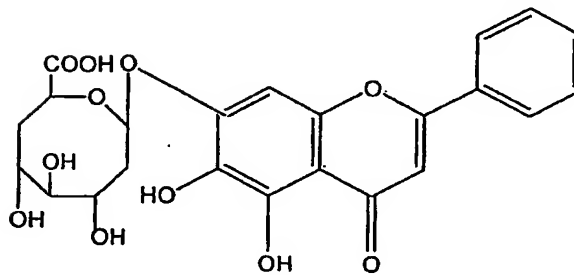
Active compounds of the present invention further comprise a structure according to the following formulas:

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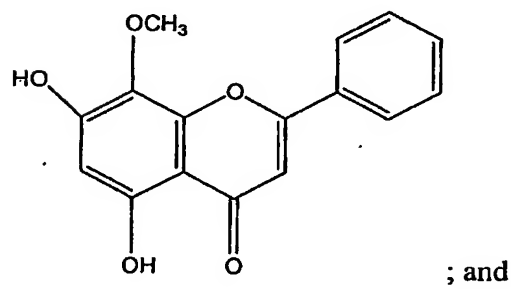
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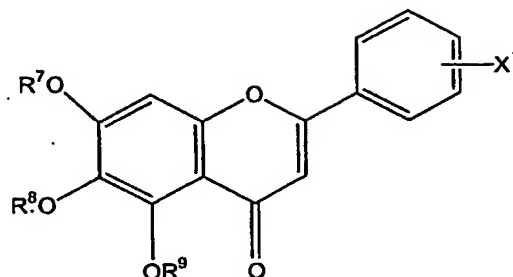


(IV)



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(V)



wherein:

R^7 , R^8 , and R^9 are each independently selected from the group consisting of H, alkyl, carbohydrates, and benzyl;

or R^7 and R^8 together are heterocycles;

or R^8 and R^9 together are heterocycles;

X^1 is selected from the group consisting of H, C, NH_2 , $NHCOCH_3$, NO_2 , and OR^{10} , wherein R^{10} is selected from the group consisting of H, alkyl, carbohydrates, and benzyl; or pharmaceutically acceptable salts thereof, and X^1 may be attached in either an ortho, meta, or para relationship.

R^7 , R^8 , and R^9 can be a moiety selected to confer water solubility to the compound to provide a novel, water-soluble compound. In addition to the description above, R^7 , R^8 , and R^9 can be a sulphate, sulphonate, phosphate, or phosphonate as selected to provide a novel, water-soluble compound. Moreover, the sulphate, sulphonate, phosphate, or phosphonate can be in the form of a water-soluble salt. As a representative non-limiting example, a water-soluble salt can be formed using an alkali metal salt, such as sodium, potassium, or ammonium. The water-soluble salt may be in the form of a mono, di-, or tri- alkali metal salt. In some embodiments, R^7 , R^8 , and R^9 can be SO_3H or PO_3H_2 and salts thereof, including, but not limited to, sodium and potassium.

Active compounds of the present invention further comprise known water-soluble derivatives of baicalein including, but not limited to, baicalein-6-sulfate, baicalein-6,7 disulfate, baicalein-6-phosphate, baicalein-6,7-diphosphate, baicalein-5,6,7-triphosphate, and pharmaceutically acceptable salts thereof, as disclosed in

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Nagai H, et al. Inhibition of hypersensitivity reactions by soluble derivatives of baicalein. *Jpn J Pharmacol* Dec; 25(6):763-7 (1975); Nohara, A. et al. Takeda Kenkyushoho 30(4): 677-81(1971); and German Patent Numbers DE1802569C3, DE1802569C3, and DE 1802569A.

A. Synthesis of Novel Compounds

Variations on the following general synthetic methods will be readily apparent to those skilled in the art and are deemed to be within the scope of the present invention.

Figure 1 shows the structures of baicalein analogs. Baicalein analogs as shown in formula (1) are obtained from a readily available commercial source or by derivitization of baicalein or commercially available baicalein analogs by methods known in the literature. Treatment of the analogs as shown in formula (1) (Phadke, P.S.; Rao, A.V. R.; Venkataraman, K. *Indian J. Chem.* 1967, 5, 131-3) with Pd-C provides baicalein analogs as shown in formula (2) (Beutler, JA; Hamel, E. *et al*, *J. Med. Chem.* 1998, 41, 2333-38). Alkylation of baicalein analogs as shown in formula (2) in the presence of a base (e.g., K_2CO_3) or tertiary amine (e.g., Et_3N) reacted with $W(CH_2)_nOR$ wherein W is a leaving group and R is H, alkyl, alkenyl, alkynyl, or carbohydrate, provides baicalein analogs as shown in formula (3). Baicalein analogs as shown in (3) are treated with $MsCl$ and reacted with an amine to provide baicalein analogs as shown in (4).

B. Pharmaceutically acceptable salts

The term "active agent" as used herein, includes the pharmaceutically acceptable salts of the compound. Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects. Examples of such salts are (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid,

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tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (b) salts formed from elemental anions such as chlorine, bromine, and iodine. In particular embodiments, pharmaceutically acceptable salts are formed with hydrochloric acid. In other particular embodiments, pharmaceutically acceptable salts are formed with malic acid.

Active agents used to prepare compositions for the present invention may alternatively be in the form of a pharmaceutically acceptable free base of active agent. Because the free base of the compound is less soluble than the salt, free base compositions are employed to provide more sustained release of active agent to the target area. Active agent present in the target area which has not gone into solution is not available to induce a physiological response, but serves as a depot of bioavailable drug which gradually goes into solution.

C. Pharmaceutical Formulations

The flavonoids and flavonoid analogs of the present invention are useful as pharmaceutically active agents and may be utilized in bulk form. More preferably, however, these compounds are formulated into pharmaceutical formulations for administration. Any of a number of suitable pharmaceutical formulations may be utilized as a vehicle for the administration of the compounds of the present invention.

It will be appreciated that certain compounds of the above Formulae can possess an asymmetric carbon atom(s) and are thus capable of existing as enantiomers. Unless otherwise specified, this invention includes such enantiomers, including racemates. The separate enantiomers may be synthesized from chiral starting materials, or the racemates can be resolved by procedures that are well known in the art of chemistry such as chiral chromatography, fractional crystallization of diastereometric salts and the like.

The compounds of the present invention may be formulated for administration for the treatment of a variety of conditions. In the manufacture of a pharmaceutical formulation according to the invention, the compounds of the present invention and the physiologically acceptable salts thereof, or the acid derivatives of either

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(hereinafter referred to as the "active compound") are typically admixed with, *inter alia*, an acceptable carrier. The carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the formulation and must not be deleterious to the patient. The carrier may be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose formulation, for example, a tablet, which may contain from 0.5% to 95% by weight of the active compound. In one particular embodiment, a pharmaceutical composition comprises less than 80% by weight of active compound. In other particular embodiments, a pharmaceutical composition comprises less than 50% by weight of active compound. One or more of each of the active compounds may be incorporated in the formulations of the invention, which may be prepared by any of the well-known techniques of pharmacy consisting essentially of admixing the components, optionally including one or more accessory ingredients.

The formulations of the invention include those suitable for oral, rectal, topical, buccal (e.g., sub-lingual), parenteral (e.g., subcutaneous, intramuscular, intradermal, or intravenous) and transdermal administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular active compound which is being used.

Formulations suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges, tablets, dragees, or syrups each containing a predetermined amount of the active compound; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of pharmacy which includes the step of bringing into association the active compound and a suitable carrier (which may contain one or more accessory ingredients as noted above).

In general, the formulations of the invention are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet may be prepared by compressing or molding a powder or granules containing the

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active compound, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the compound in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Molded tablets may be made by molding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Formulations suitable for buccal (sub-lingual) administration include lozenges comprising the active compound in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Formulations of the present invention suitable for parenteral administration conveniently comprise sterile aqueous preparations of the active compound, which preparations are preferably isotonic with the blood of the intended recipient. These preparations may be administered by means of subcutaneous, intravenous, intramuscular, or intradermal injection. Such preparations may conveniently be prepared by admixing the compound with water or a glycine buffer and rendering the resulting solution sterile and isotonic with the blood.

Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing the active compound with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Formulations suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil.

Formulations suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Formulations suitable for transdermal administration may also be delivered by iontophoresis (*see, for example, Pharmaceutical Research* 3(6):318 (1986)) and typically take the form of an optionally buffered aqueous solution of the active compound. Suitable formulations comprise citrate or bis/tris buffer (pH 6) or ethanol/water and contain from 0.01 to 0.2M active ingredient.

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The present invention may also be formulated into a sustained-release preparation. A sustained-release composition includes, but is not limited to, those in which the active ingredient is bound to an ion exchange resin which is optionally coated with a diffusion barrier to modify the release properties of the resin.

Carriers and/or diluents which may be used include vaseline, lanoline, glycerin, vegetable oils, or fat emulsions, polyethylene glycols, alcohols, transdermal enhancers, natural or hardened oils or waxes, and combinations of two or more thereof.

In particular embodiments, the present invention provides compounds of the formulas described herein that are water soluble. In some embodiments, water solubility is conferred to the compounds described herein by preparation of a water-soluble salt thereof. As a non-limiting example, alkali metal salts such as sodium, potassium, or ammonium can be used to confer water solubility to the compounds. The water-soluble salt may be in the form of a mono, di-, or tri- alkali metal salt. In some embodiments, a moiety capable of conferring water solubility to the compound to provide a novel, water-soluble compound can be added. Such moieties include, but are not limited to, sulphate, sulphonate, phosphate, or phosphonate moieties as selected to provide a novel, water-soluble compound. Moreover, the sulphate, sulphonate, phosphate, or phosphonate can be in the form of a water-soluble salt as described above. In some embodiments, the moiety capable of conferring water solubility to the compound can be SO_3H or PO_3H_2 and salts thereof, including, but not limited to, sodium and potassium. In some embodiments, the active compounds of the present invention comprise known water-soluble derivatives of baicalein including, but not limited to, baicalein-6-sulfate, baicalein-6,7-disulfate, baicalein-6-phosphate, baicalein-6,7-diphosphate, baicalein-5,6,7-triphosphate, and pharmaceutically acceptable salts thereof.

D. Methods of Use

In addition to the compounds of the formulas described herein, the present invention also provides useful therapeutic methods. For example, the present

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invention provides a method of treating septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders.

In particular embodiments, organ damage includes, but is not limited to, liver damage, kidney damage, and lung damage.

In particular embodiments, neurodegenerative diseases include, but are not limited to, Parkinson's disease, Alzheimer's disease, cognition deficit, memory loss, and stroke.

In other particular embodiments, exemplary cancers include, but are not limited to, leukemia, lymphoma, colon cancer, renal cancer, liver cancer, breast cancer, lung cancer, prostate cancer, ovarian cancer, melanoma, small cell lung cancer, testicular cancer, esophageal cancer, stomach cancer, endometrial cancer, central nervous system cancer, and the like. The term "cancer" has its understood meaning in the art, for example, an uncontrolled growth of tissue that has the potential to spread to distant sites of the body (*i.e.*, metastasize). Preferred are methods of treating and preventing tumor-forming cancers. The term "tumor" is also understood in the art, for example, as an abnormal mass of undifferentiated cells within a multicellular organism. Tumors can be malignant or benign. Preferably, the inventive compounds and methods disclosed herein are used to prevent and treat malignant tumors.

In still yet other particular embodiments, cardiac disorders include, but are not limited to, cardiac ischemia, congestive heart failure, and hypertension.

Suitable subjects to be treated according to the present invention include both avian and mammalian subjects, preferably mammalian. Mammals according to the present invention include but are not limited to canine, felines, bovines, caprines, equines, ovines, porcines, rodents (*e.g.* rats and mice), lagomorphs, primates, and the like, and encompass mammals *in utero*. Humans are preferred.

Illustrative avians according to the present invention include chickens, ducks, turkeys, geese, quail, pheasant, ratites (*e.g.*, ostrich) and domesticated birds (*e.g.*, parrots and canaries), and include birds *in ovo*. Chickens and turkeys are preferred.

Any mammalian subject in need of being treated according to the present invention is suitable. Human subjects are preferred. Human subjects of both genders

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and at any stage of development (*i.e.*, neonate, infant, juvenile, adolescent, adult) can be treated according to the present invention.

As noted above, the present invention provides pharmaceutical formulations comprising the compounds of formulae described herein, or pharmaceutically acceptable salts thereof, in pharmaceutically acceptable carriers for any suitable route of administration, including but not limited to, oral, rectal, topical, buccal, parenteral, intramuscular, intradermal, intravenous, and transdermal administration.

According to the present invention, methods of this invention comprise administering an effective amount of a composition of the present invention as described above to the subject. The effective amount of the composition, the use of which is in the scope of present invention, will vary somewhat from subject to subject, and will depend upon factors such as the age and condition of the subject and the route of delivery. Such dosages can be determined in accordance with routine pharmacological procedures known to those skilled in the art. For example, the compounds of the present invention can be administered to the subject in an amount ranging from a lower limit from about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, or 10% to an upper limit ranging from about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% by weight of the composition. In some embodiments, the compounds comprise from about 0.05 to about 95% by weight of the composition. In other embodiments, the compounds comprise from about 0.05 to about 60% by weight of the composition. In still other embodiments, the compounds comprise from about 0.05 to about 10% by weight of the composition.

The therapeutically effective dosage of any specific compound will vary somewhat from compound to compound, patient to patient, and will depend upon the condition of the patient and the route of delivery. As a general proposition, a dosage from about 0.1 to about 50 mg/kg will have therapeutic efficacy, with still higher

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dosages potentially being employed for oral and/or aerosol administration. Toxicity concerns at the higher level may restrict intravenous dosages to a lower level such as up to about 10 mg/kg, all weights being calculated based upon the weight of the active base, including the cases where a salt is employed. Typically a dosage from about 0.5 mg/kg to about 5 mg/kg will be employed for intravenous or intramuscular administration. A dosage from about 10 mg/kg to about 50 mg/kg may be employed for oral administration.

In particular embodiments, compounds of the present invention may be administered at a daily dosage of from about 0.1 mg to about 20 mg per kg of animal body weight, which can be given in divided doses q.d. to q.i.d. or in a sustained release form. For humans, the total daily dose may be in the range of from about 5 mg to about 1,400 mg, and in other particular embodiments, the total daily dose is in the range of from about 10 mg to about 100 mg. In still other embodiments, the unit dosage forms suitable for oral administration may comprise about 2 mg to about 1,400 mg of the compound optionally admixed with a solid or liquid pharmaceutical carrier or diluent. The compounds of the present invention can be administered in any amount appropriate to administer to the subject for treatment of the condition desired to be treated as determined by one of ordinary skill in the art by reference to the pertinent texts and literature and/or by using routine experimentation. (See, for example, Remington, *The Science And Practice of Pharmacy* (9th Ed. 1995).

The present invention is explained in greater detail in the following non-limiting examples.

EXAMPLE 1

Preparation of Novel Baicalein Analogs

Treatment of the analogs as shown in formula (1) with Pd-C provides baicalein analogs as shown in formula (2). Alkylation of baicalein analogs as shown in formula (2) in the presence of a base (e.g., K_2CO_3) or tertiary amine (e.g., Et_3N) reacted with $W(CH_2)_nOR$ wherein W is a leaving group and R is H, alkyl, alkenyl, alkynyl, or carbohydrate, provides baicalein analogs as shown in formula (3).

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Baicalein analogs as shown in (3) are treated with MsCl and reacted with an amine to provide baicalein analogs as shown in (4).

Preparation of 4'-(2-hydroxyethylamino)-5,7-dihydroxy-6-methoxyflavone. A mixture of the aminobaicalein (1.5 g, 5 mmol) [(2), R1,R3 = H, R2 = OMe) NH2 at 4'-position; Phadke, P.S.; Rao, A.V. R.; Venkataraman, K. *Indian J. Chem.* 1967, 5, 131-3], 2-bromoethanol (749 mg, 6 mmol), and anhyd. potassium carbonate (1 g) in acetone (20 ml) is refluxed overnight. The reaction mixture is cooled, the solid filtered off, washed with more acetone, and the filtrate is evaporated to give a residue. The residue is treated with a small amount of ether to remove excess of solid 2-bromoethanol to give a solid (3), the expected product.

Preparation of 4'-(2-methanesulfonatoethylamino)-5,7-dihydroxy-6-methoxyflavone. A solution of 4'-(2-hydroxy-ethylamino)-5,7-dihydroxy-6-methoxyflavone (1.72 g, 5 mmol) in methylene chloride (40 ml) at 0 °C under nitrogen is treated with mesyl chloride (801 mg, 10 mmol) dropwise followed by triethylamine (1.518 g, 15 mmol). The reaction mixture is kept at 0 °C for 1 h, warmed up to RT for 1 hr, and then poured into an ice-water mixture. The organic layer is separated and the aqueous layer is extracted with methylene chloride twice (40 ml x 2). The organic layers are combined, washed with water followed by saturate brine and dried (MgSO4), thus giving the expected mesylate upon evaporation of the dried solution.

Preparation of 4'-[2-(N,N-diethylamino)ethylamino]-5,7-dihydroxy-6-methoxyflavone. A solution of the mesylate obtained from B) (442 mg, 1 mmol), diethylamine (1 ml) and anhyd. THF (20 ml) is heated under reflux overnight. The reaction is cooled and evaporated to give the title compound.

Preparation of 6,7-methylenedioxy-5-hydroxy-4'-methoxy-flavone. A solution of Kanzakiflavone-1 or 6,7-methylenedioxy-5,4'-dihydroxyflavone (1.49 g, 5 mmol) [Compound (4): R1 and R2 = CH2, X(CH2)nNR1R2 = OH; Manchanda, V. P.; Khanna, R. N. *Curr. Sci.* (1977), 46(13), 445-6.] and dimethylsulfate (1.02 g, 8 mmol) in acetone (20 ml) is refluxed in the presence of anhyd. potassium carbonate (0.5 g) overnight. The reaction mixture is filtered off and the filtrate is evaporated to

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give a dark brown residue. The residue is taken up in ethyl acetate and washed with water followed by saturated brine solution and dried (MgSO₄), thus giving the title compound, upon filtration and evaporation.

Preparation of 4'-[2-(N,N-diethylamino)ethoxy]-6,7-methylenedioxy-5-hydroxy-flavone. Similar to the preparation of 4'-[2-(N,N-diethylamino)ethylamino]-5,7-dihydroxy-6-methoxyflavone (discussed above), the title compound is prepared starting from 6,7-methylenedioxy-5,4'-dihydroxyflavone.

EXAMPLE 2

Effects of Baicalein on Blood Pressure, Heart Rate, and Mortality

Materials and Methods. Male Charles River Wistar-Kyoto rats (230-300 g) from Japan were used. The rats were anesthetized by intraperitoneal injection of urethane (1.2 g/kg). The trachea was cannulated to facilitate respiration and rectal temperature was maintained at 37°C with a homeothermic blanket (Harvard Apparatus, South Natick, MA). The right carotid artery was cannulated and connected to a pressure transducer (P233ID, Statham, Oxnard, CA) for the measurement of phasic and mean arterial pressure as well as heart rate which were displayed on a Gould model TA5000 polygraph recorder (Gould, Valley View, OH). The left jugular vein was cannulated for the administration of drugs. Upon completion of the surgical procedure, cardiovascular parameters were allowed to stabilize for 20 min. After recording of the baseline hemodynamic parameters, the animals were given vehicle (DMSO) or baicalein (5, 10, or 20 mg/kg, i.v.). Baicalein was administered intravenously at 1 hour after lipopolysaccharide (LPS) administration. Prior to (i.e., at time 0) and at every hour after vehicle or LPS, 0.5 ml of blood was taken to measure the changes in plasma levels of TNF- α and nitrate (an indicator of NO formation (Yen, M.H. et al., *Shock* 14, 60-67, 2000). Any blood withdrawn was immediately replaced by the injection of an equal volume of saline.

Plasma TNF- α Determination. The plasma samples (100 μ l) were diluted 1:2 and TNF- α was measured in duplicate with an enzyme linked immunoadsorbent

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assay (ELISA) kit (Genzyme, Cambridge, MA) (Yen, M.H. et al., *Biochem. Biophys. Res. Commun.* 228:459-466, 1996).

Superoxide Anion Detection by Chemiluminescence. Detection of superoxide was performed as previously described. Briefly, the aortas were cut into 5-mm rings and then incubated in Krebs-HEPES buffer containing 0.25 mmol/L lucigenin. Counts were obtained at 15-minute intervals at 37°C with a luminescence measurement system (microLumate plus LB96V, EG&G Berthold).

Plasma Nitrate Determination. Determination of plasma nitrate was performed as previously described. Briefly, for the reduction of liquid nitrate to the gas NO, 10 µl was injected into a collection chamber containing 5% VCl₃. NO was carried by a constant stream of helium gas into a NO analyzer (Seivers 270B NOA, Seivers Instruments Inc.).

iNOS Detection by Western Blotting. Detection of inducible nitric oxide synthase (iNOS) by western blotting was performed as described previously. The primary antibodies probed in the experiment were mouse anti-iNOS (Transduction Laboratories).

Results and Discussion. *In vivo* results demonstrated that following LPS administration (10 mg/kg, i.v.) in rats, the mean blood pressure decreased from 80 ± 12 mmHg to 21 ± 10 mmHg and heart rate increased from 285 ± 25 beats/min to 418 ± 15 beats/min induced by LPS (10 mg/kg, i.v.), respectively. In contrast, the treatment of baicalein (20 mg/kg, i.v. in rats) significantly improved mean blood pressure from 21 ± 10 mmHg to 76 ± 13 mmHg and heart rate from 418 ± 15 beats/min to 342 ± 21 beats/min, respectively. Moreover, baicalein (60 mg/kg in 3 divided doses i.p. in ICR mice; each dose injected at 2 hr post dose and then 8hr postdose and at 8-hour intervals) significantly reduced the mortality rate from 67 % to 16 % of ICR mice within 24 hours. Likewise in rats, baicalein also reduced superoxide formation from 800 pmol/15 min/mg to 510 pmol/15 min/mg and suppressed iNOS expression in aortic tissue-induced by LPS. Furthermore, baicalein also decreased plasma TNF-α levels from 1700 ng/ml to 850 ng/ml at 2 hr and reduced plasma nitrate levels from 242 µM to 85 µM at 6 hr. With LPS-rat aortic ring preparation *ex vivo*, the dose-response curves for norepinephrine (NE), acetylcholine

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(ACh), and L-arginine were significantly improved following post-treatment with baicalein. In addition, baicalein also significantly inhibited $I_{\kappa}B\alpha$ degradation and iNOS expression in aortas from LPS rats.

While not wishing to be bound to any particular theory, we propose that baicalein and analogs thereof may provide anti-sepsis effects through inactivation of $NF_{\kappa}B$ pathway.

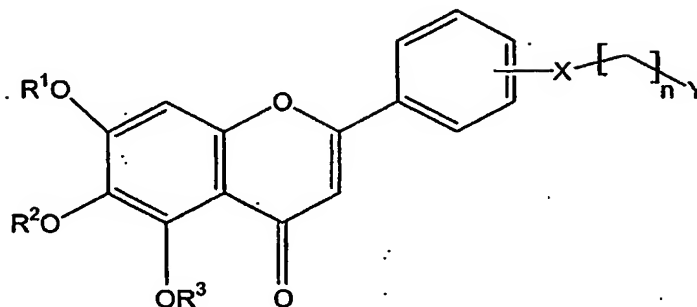
In conclusion, we have studied the activity of baicalein and propose a number of baicalein analogs. We have evaluated the activity of baicalein as an agent to produce anti-sepsis effects. The studies revealed that baicalein improves LPS-induced septic shock and mortality.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

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What is claimed is:

1. A compound according to formula I:



wherein:

R^1 , R^2 , and R^3 are each independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, and carbohydrates;

or R^1 and R^2 together are heterocycles;

or R^2 and R^3 together are heterocycles;

X is selected from the group consisting of H, C, N, NR' , $NR'R''$, $NR'SO_2R''$, O, and S, subject to the proviso that the compound according to formula I is not baicalein, and wherein R' and R'' are each independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, and carbohydrates; and wherein when X is $NR'R''$ or $NR'SO_2R''$, n is 0 and Y is not present, and R' and R'' together form a 5 to 7-membered ring;

n is from 0 to 3; and

Y is selected from the group consisting of OR^4 and NR^5R^6 , wherein R^4 , R^5 , and R^6 are each independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, and carbohydrates, and R^5 and R^6 together may form a 5 to 7-membered ring; or pharmaceutically acceptable salts thereof.

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2. The compound according to claim 1, wherein R^1 , R^2 , and R^3 are each independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, and carbohydrates, subject to the proviso that when X is H, R^1 , R^2 , and R^3 are not all H.
3. The compound according to claim 2, wherein the alkyl group is a C1-C3 alkyl.
4. The compound according to claim 2, wherein the carbohydrate is selected from the group consisting of monosaccharides, oligosaccharides, and polysaccharides.
5. The compound according to claim 4, wherein the carbohydrate is a monosaccharide.
6. The compound according to claim 4, wherein the carbohydrate is a disaccharide.
7. The compound according to claim 1, wherein R^1 and R^2 together are heterocycles.
8. The compound according to claim 7, wherein R^1 and R^2 together is a five-membered ring structure.
9. The compound according to claim 7, wherein R^1 and R^2 together is a six-membered ring structure.
10. The compound according to claim 1, wherein R^2 and R^3 together are heterocycles.
11. The compound according to claim 10, wherein R^2 and R^3 together is a five-membered ring structure.

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12. The compound according to claim 10, wherein R^2 and R^3 together is a six-membered ring structure.
13. The compound according to claim 1, wherein X is N.
14. The compound according to claim 1, wherein X is O.
15. The compound according to claim 1, wherein X is substituted on the ortho position of the phenyl ring.
16. The compound according to claim 1, wherein X is substituted on the meta position of the phenyl ring.
17. The compound according to claim 1, wherein X is substituted on the para position of the phenyl ring.
18. The compound according to claim 1, wherein Y is OR^4 .
19. The compound according to claim 1, wherein Y is NR^5R^6 .
20. The compound according to claim 1, wherein the compound is water-soluble.
21. A pharmaceutical formulation comprising a compound according to claim 1 and at least one pharmaceutically acceptable carrier, diluent, or excipient.
22. A pharmaceutical formulation comprising a compound according to claim 21, wherein the pharmaceutically acceptable carrier is an aqueous carrier.
23. A method of treating septic shock, comprising administering to a subject in need thereof an effective amount of a compound according to claim 1.

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24. A method of treating inflammation, comprising administering to a subject in need thereof, an effective amount of a compound according to claim 1.

25. A method of treating organ damage, comprising administering to a subject in need thereof an effective amount of a compound according to claim 1.

26. The method according to claim 25, wherein the organ damage is liver damage.

27. The method according to claim 25, wherein the organ damage is kidney damage.

28. A method of treating neurodegenerative diseases selected from the group consisting of Parkinson's disease, Alzheimer's disease, cognition deficit, memory loss, and stroke, comprising administering to a subject in need thereof an effective amount of a compound according to claim 1.

29. A method of treating cancer, comprising administering to a subject in need thereof, an effective amount of a compound according to claim 1.

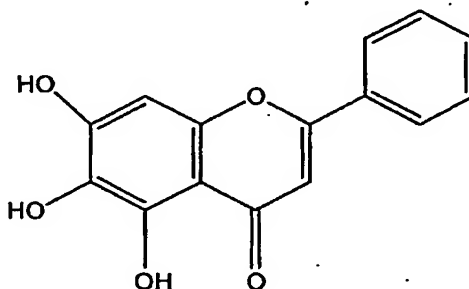
30. The method according to claim 29, wherein the cancer is selected from the group consisting of skin cancer, small cell lung cancer, testicular cancer, esophageal cancer, breast cancer, endometrial cancer, ovarian cancer, central nervous system cancer, liver cancer, and prostate cancer.

31. A method of treating cardiac disorders selected from the group consisting of cardiac ischemia, congestive heart failure, and hypertension, comprising administering to a subject in need thereof an effective amount of a compound according to claim 1.

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32. A method of treating conditions selected from the group consisting of septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders, comprising administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of a compound of the formula II:

(II)



33. The method according to claim 32, wherein the organ damage is liver damage.

34. The method according to claim 32, wherein the organ damage is kidney damage.

35. The method according to claim 32, wherein the neurodegenerative diseases are selected from the group consisting of Parkinson's disease, Alzheimer's disease, cognition deficit, memory loss, and stroke.

36. The method according to claim 32, wherein the cancer is selected from the group consisting of skin cancer, small cell lung cancer, testicular cancer, esophageal cancer, breast cancer, endometrial cancer, ovarian cancer, central nervous system cancer, liver cancer, and prostate cancer.

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37. The method according to claim 32, wherein the cardiac disorders are selected from the group consisting of cardiac ischemia, congestive heart failure, and hypertension.

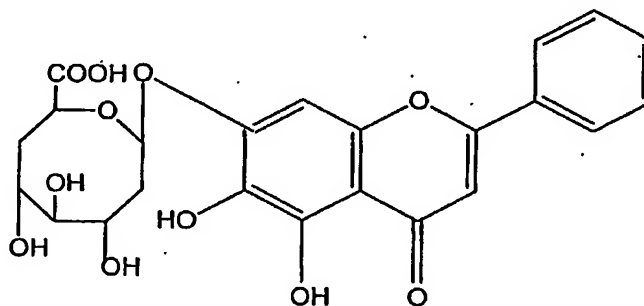
38. The method according to claim 32, wherein the pharmaceutical composition further comprises at least one other compound useful in the prevention or treatment of septic shock.

39. The method according to claim 32, wherein the pharmaceutical composition is administered orally.

40. The method according to claim 32, wherein the pharmaceutical composition is administered parenterally.

41. A method of treating conditions selected from the group consisting of septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders, comprising administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of a compound of the formula III:

(III)



42. The method according to claim 41, wherein the organ damage is liver damage.

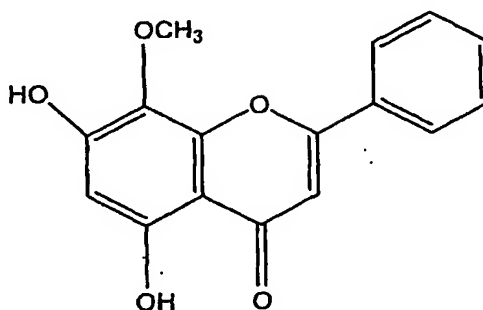
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43. The method according to claim 41, wherein the organ damage is kidney damage.
44. The method according to claim 41, wherein the neurodegenerative diseases are selected from the group consisting of Parkinson's disease, Alzheimer's disease, cognition deficit, memory loss, and stroke.
45. The method according to claim 41, wherein the cancer is selected from the group consisting of skin cancer, small cell lung cancer, testicular cancer, lymphoma, leukemia, esophageal cancer, stomach cancer, colon cancer, breast cancer, endometrial cancer, ovarian cancer, central nervous system cancer, liver cancer, and prostate cancer.
46. The method according to claim 41, wherein the cardiac disorders are selected from the group consisting of cardiac ischemia, congestive heart failure, and hypertension.
47. The method according to claim 41, wherein the pharmaceutical composition further comprises at least one other compound useful in the prevention or treatment of septic shock.
48. The method according to claim 41, wherein the pharmaceutical composition is administered orally.
49. The method according to claim 41, wherein the pharmaceutical composition is administered parenterally.
50. A method of treating conditions selected from the group consisting of septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders, comprising administering to a subject in need thereof, a

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pharmaceutical composition comprising a therapeutically effective amount of a compound of the formula IV:

(IV)



51. The method according to claim 50, wherein the organ damage is liver damage.
52. The method according to claim 50, wherein the organ damage is kidney damage.
53. The method according to claim 50, wherein the neurodegenerative diseases are selected from the group consisting of Parkinson's disease, Alzheimer's disease, cognition deficit, memory loss, and stroke.
54. The method according to claim 50, wherein the cancer is selected from the group consisting of skin cancer, small cell lung cancer, testicular cancer, lymphoma, leukemia, esophageal cancer, stomach cancer, colon cancer, breast cancer, endometrial cancer, ovarian cancer, central nervous system cancer, liver cancer, and prostate cancer.
55. The method according to claim 50, wherein the cardiac disorders are selected from the group consisting of cardiac ischemia, congestive heart failure, and hypertension.

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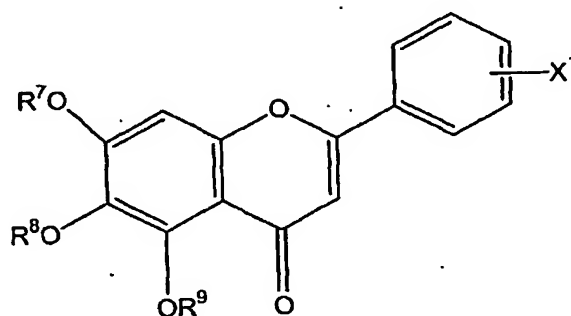
56. The method according to claim 50, wherein the pharmaceutical composition further comprises at least one other compound useful in the prevention or treatment of septic shock.

57. The method according to claim 50, wherein the pharmaceutical composition is administered orally.

58. The method according to claim 50, wherein the pharmaceutical composition is administered parenterally.

59. A method of treating conditions selected from the group consisting of septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders, comprising administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of a compound of the formula V:

(V)



wherein:

R^7 , R^8 , and R^9 are each independently selected from the group consisting of H, alkyl, carbohydrates, and benzyl;

or R^7 and R^8 together are heterocycles;

or R^8 and R^9 together are heterocycles;

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X^1 is selected from the group consisting of H, C, NH_2 , $NHCOCH_3$, NO_2 , and OR^{10} , wherein R^{10} is selected from the group consisting of H, alkyl, carbohydrates, and benzyl; or pharmaceutically acceptable salts thereof.

60. The method according to claim 59, wherein R^7 , R^8 , and R^9 are each independently selected from the group consisting of H, alkyl, carbohydrates, and benzyl.

61. The method according to claim 60, wherein the alkyl group is C1-C3 alkyl.

62. The method according to claim 60, wherein the carbohydrate is selected from the group consisting of monosaccharides, oligosaccharides, and polysaccharides.

63. The method according to claim 60, wherein the carbohydrate is a monosaccharide.

64. The method according to claim 60, wherein the carbohydrate is a disaccharide.

65. The method according to claim 59, wherein R^7 and R^8 together are heterocycles.

66. The method according to claim 65, wherein R^7 and R^8 together is a five-membered ring structure.

67. The method according to claim 65, wherein R^7 and R^8 together is a six-membered ring structure.

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68. The method according to claim 59, wherein R^8 and R^9 together are heterocycles.
69. The method according to claim 68, wherein R^8 and R^9 together is a five-membered ring structure.
70. The method according to claim 68, wherein R^8 and R^9 together is a six-membered ring structure.
71. The method according to claim 59, wherein X^1 is substituted on the ortho position of the phenyl ring.
72. The method according to claim 59, wherein X^1 is substituted on the meta position of the phenyl ring.
73. The method according to claim 59, wherein X^1 is substituted on the para position of the phenyl ring.
74. The method according to claim 59, wherein the organ damage is liver damage.
75. The method according to claim 59, wherein the organ damage is kidney damage.
76. The method according to claim 59, wherein the neurodegenerative diseases are selected from the group consisting of Parkinson's disease, Alzheimer's disease, cognition deficit, memory loss, and stroke.
77. The method according to claim 59, wherein the cancer is selected from

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the group consisting of skin cancer, small cell lung cancer, testicular cancer, esophageal cancer, breast cancer, endometrial cancer, ovarian cancer, central nervous system cancer, liver cancer, and prostate cancer.

78. The method according to claim 59, wherein the cardiac disorders are selected from the group consisting of cardiac ischemia, congestive heart failure, and hypertension.

79. The method according to claim 59, wherein the pharmaceutical composition further comprises at least one other compound useful in the prevention or treatment of septic shock.

80. The method according to claim 59, wherein the pharmaceutical composition is administered orally.

81. The method according to claim 59, wherein the pharmaceutical composition is administered parenterally.

82. The compound according to claim 2, wherein the alkyl is a lower alkyl.

83. The method according to claim 60, wherein the alkyl is a lower alkyl.

84. A method of treating conditions selected from the group consisting of septic shock, inflammation, organ damage, neurodegenerative diseases, cancer, and cardiac disorders, comprising administering to a subject in need thereof, a pharmaceutical composition comprising a therapeutically effective amount of a compound selected from the group consisting of baicalein-6-sulfate, baicalein-6,7-disulfate, baicalein-6-phosphate, baicalein-6,7-diphosphate, baicalein-5,6,7-triphosphate, and pharmaceutically acceptable salts thereof.

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85. The method according to claim 84, wherein the organ damage is liver damage.

86. The method according to claim 84, wherein the organ damage is kidney damage.

87. The method according to claim 84, wherein the neurodegenerative diseases are selected from the group consisting of Parkinson's disease, Alzheimer's disease, cognition deficit, memory loss, and stroke.

88. The method according to claim 84, wherein the cancer is selected from the group consisting of skin cancer, small cell lung cancer, testicular cancer, esophageal cancer, breast cancer, endometrial cancer, ovarian cancer, central nervous system cancer, liver cancer, and prostate cancer.

89. The method according to claim 84, wherein the cardiac disorders are selected from the group consisting of cardiac ischemia, congestive heart failure, and hypertension.

90. The method according to claim 84, wherein the pharmaceutical composition further comprises at least one other compound useful in the prevention or treatment of septic shock.

91. The method according to claim 84, wherein the pharmaceutical composition is administered orally.

92. The method according to claim 84, wherein the pharmaceutical composition is administered parenterally.

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BAICALEIN AND BAICALEIN ANALOGS AND USES THEREOF

Abstract of the Disclosure

The present invention relates to flavonoids, novel flavonoid analogs, and pharmaceutical formulations containing the same and methods of use thereof. Examples of flavonoids include, but are not limited to, baicalein, baicalin, wogonin, and analogs thereof. Uses of the present invention include, but are not limited to, use for the prevention and treatment of septic shock and other disorders. The compounds described herein can be water soluble.

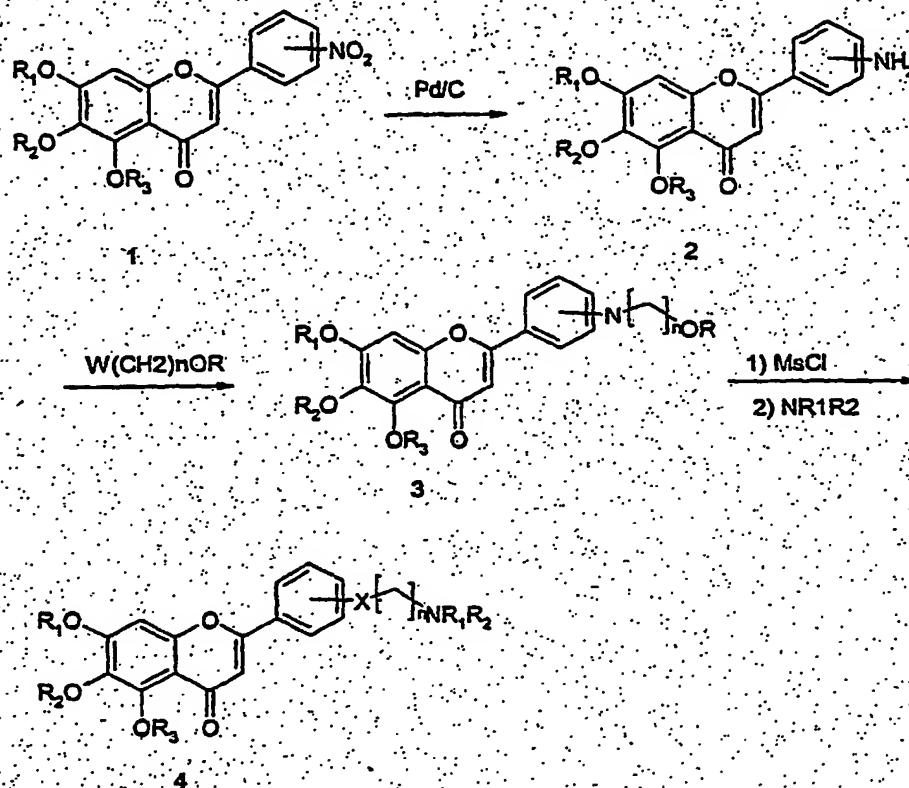


Figure 1

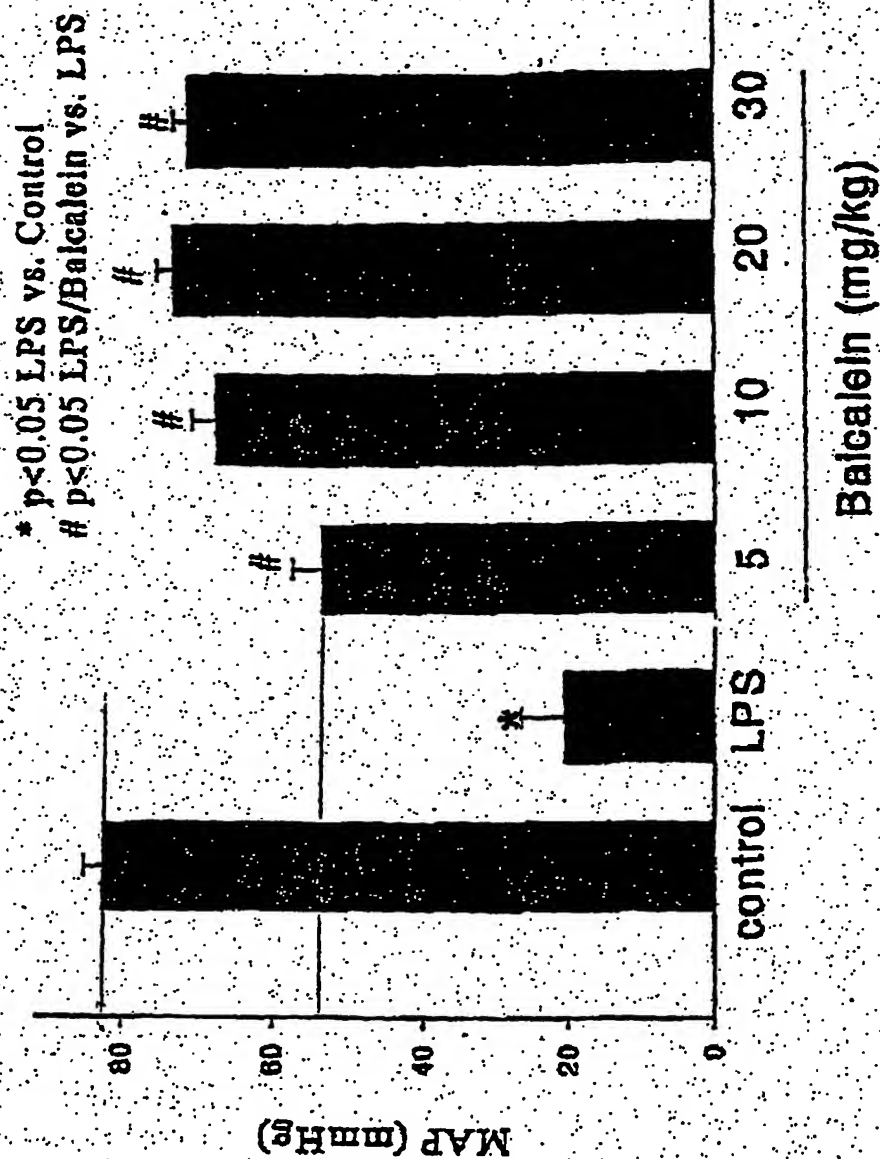


Figure 2. Effects of baicalein on mean arterial blood pressor (MAP) in rats treated with endotoxin (*E. coli* lipopolysaccharide, LPS). Data represents as means \pm S.E.M., * $p < 0.05$ (LPS vs. Control) and # $p < 0.05$ (Baicalein vs. LPS alone) ($n=8$).

* $p < 0.05$ LPS vs. Control
 # $p < 0.05$ LPS/Balcalein vs. LPS

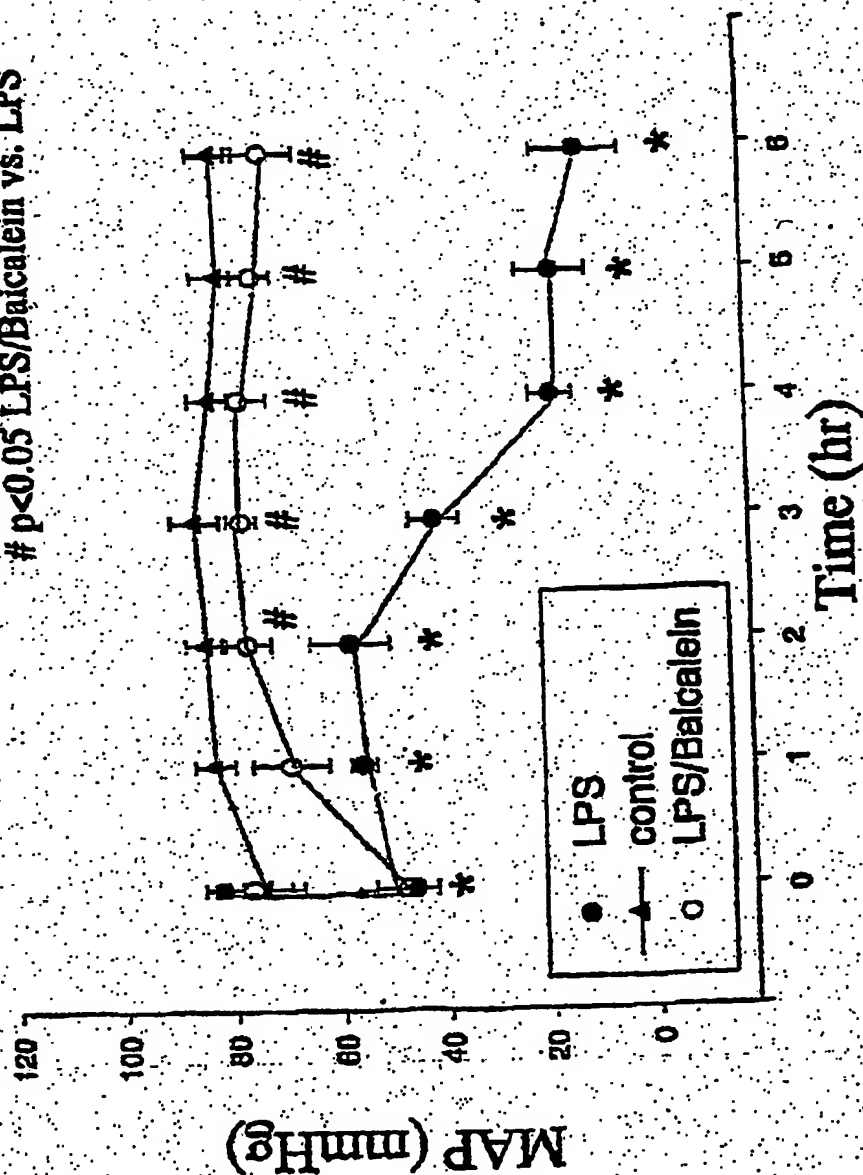


Figure 3. Effects of balcalein on the change of mean arterial blood pressor (MAP) in rats treated with endotoxin ($n=8$) (E. coli lipopolysaccharide, LPS). Data represents as means \pm S.E.M., * $p < 0.05$ (LPS vs. Control) and # $p < 0.05$ (Balcalein vs. LPS).

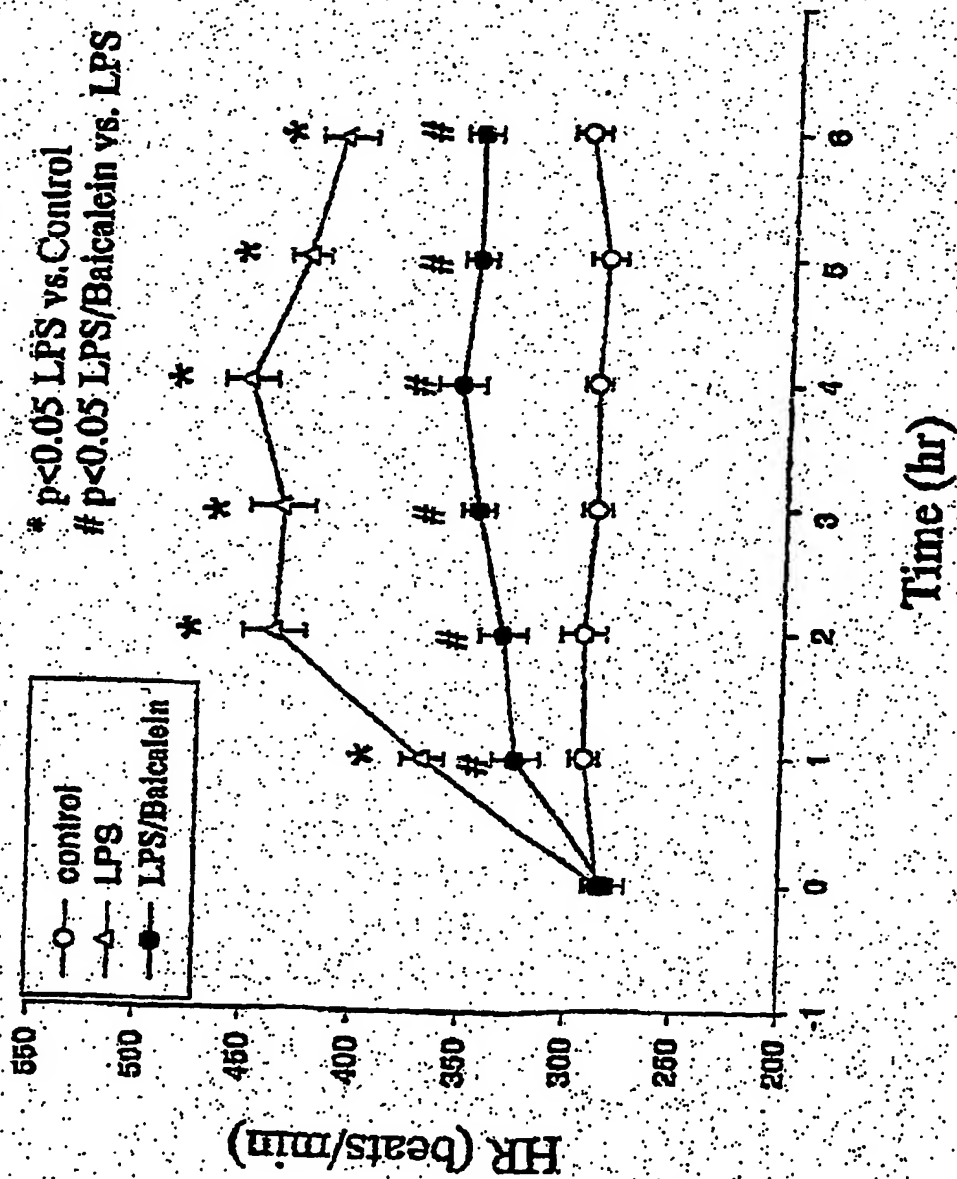


Figure 4. Effects of baicalein on the change of heart rate (HR) in rats treated with endotoxin (n=8) (E. coli lipopolysaccharide, LPS). Data represents as means \pm S.E.M., * p<0.05 (LPS vs. Control) and # p<0.05 (Baicalein vs. LPS).

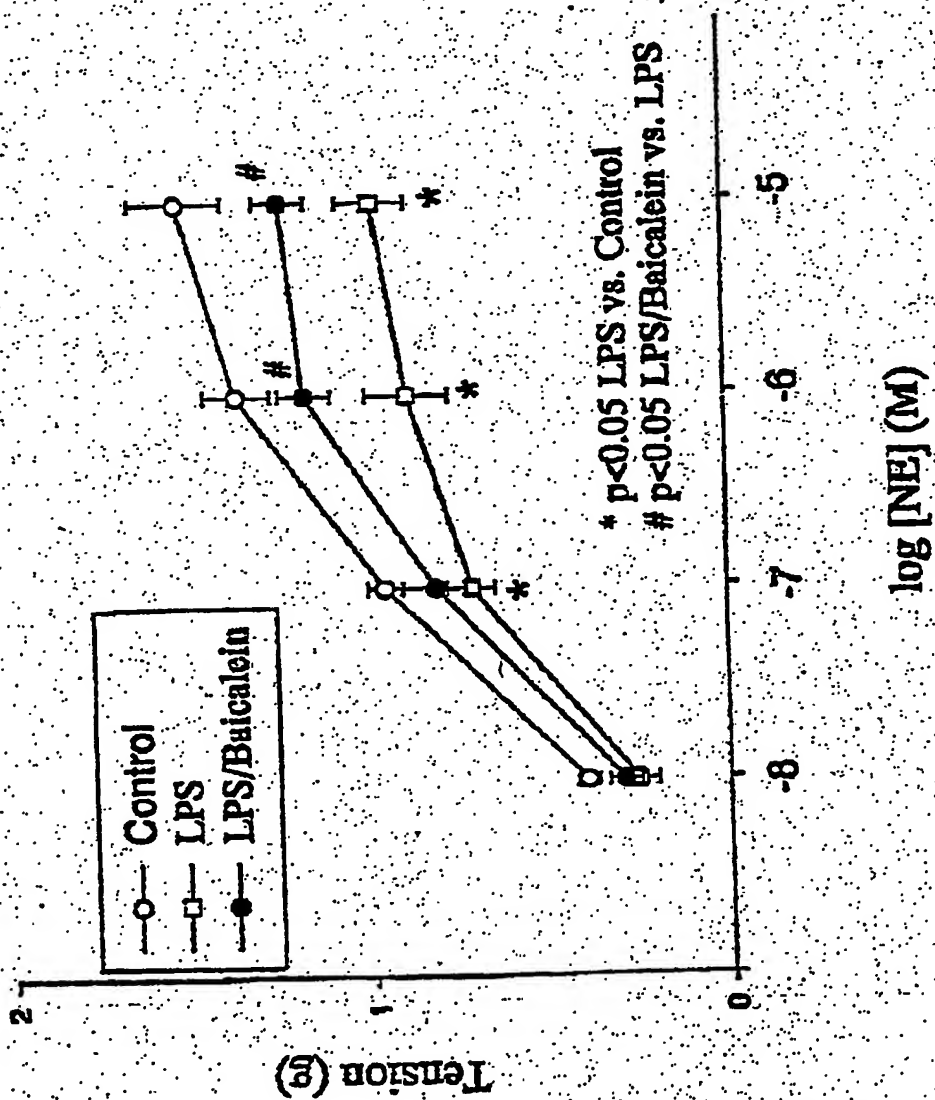


Figure 5. Effects of baicalein treatment on the concentration-response curve of NE in aortic rings from LPS-treated rats ($n=6$). Data represents as means \pm S.E.M., * $p < 0.05$ (LPS vs. Control) and # $p < 0.05$ (Baicalein vs. LPS).

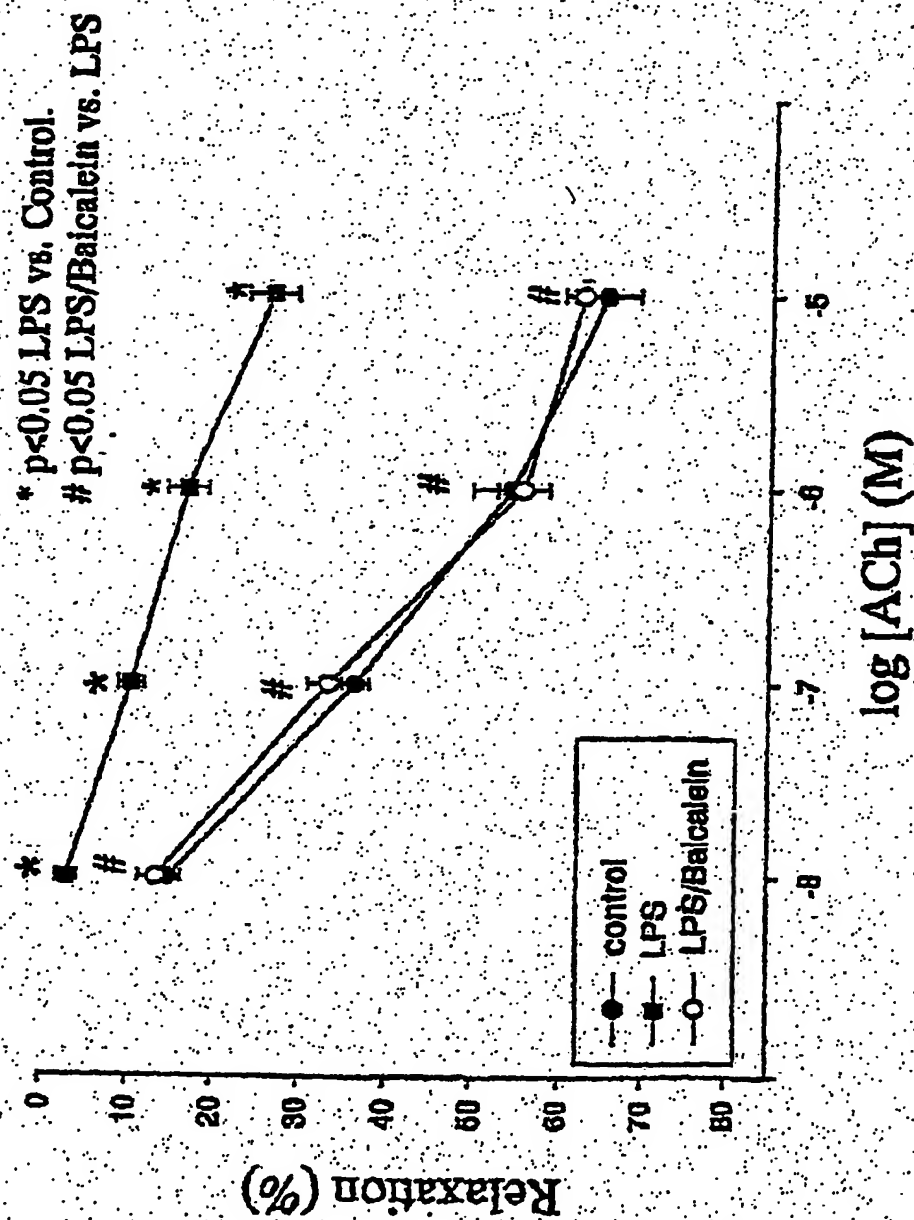


Figure 6. Effects of baicalein on the concentration-response curve of acetylcholine in aortic rings from LPS-treated rat. (n=6) Data represents as means \pm S.E.M., * $p < 0.05$ (LPS vs. Control) and # $p < 0.05$ (Baicalein vs. LPS).

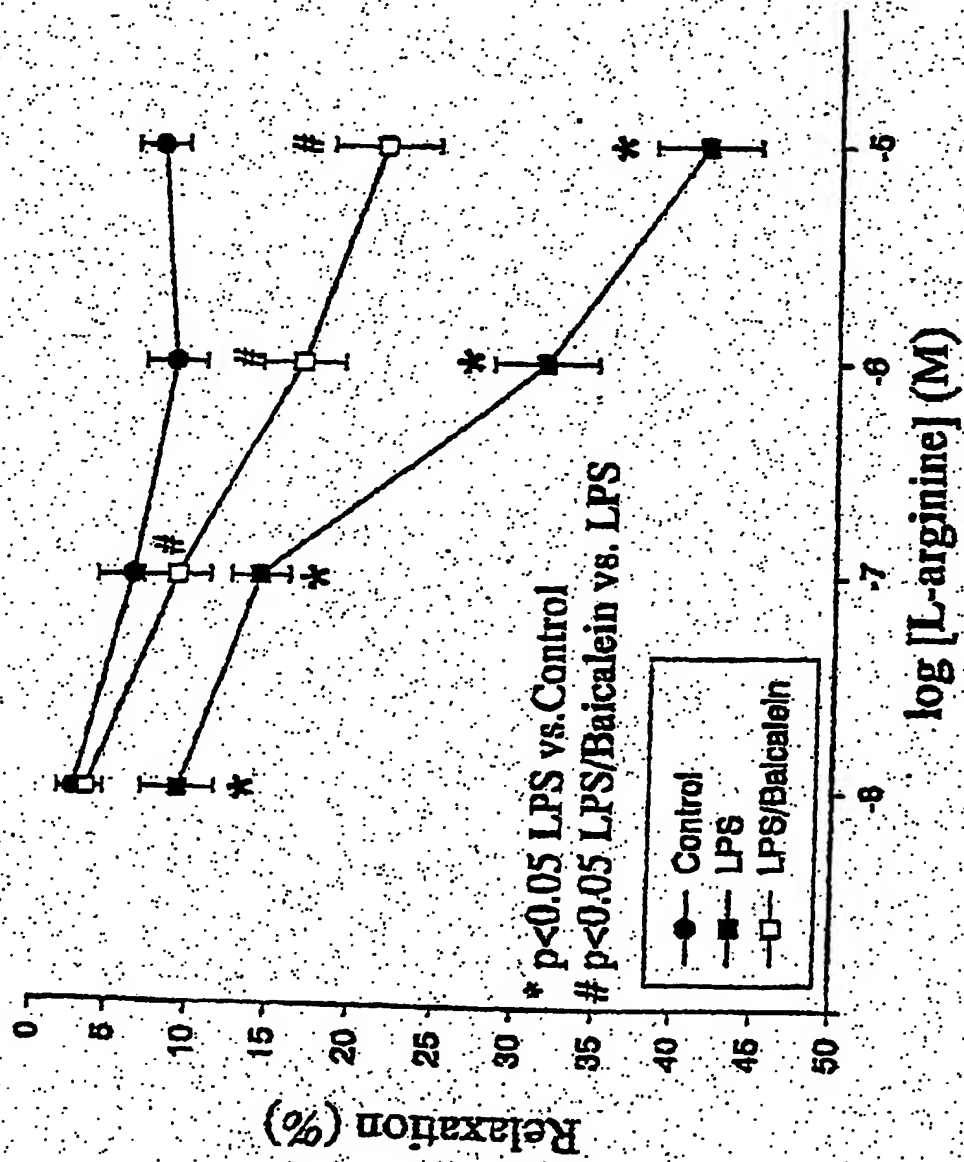


Figure 7. Effects of baicalein on the concentration - response curve of L-arginine in aortic rings from LPS-treated rat.(n=6) Data represents as means \pm S.E.M., * p<0.05 (LPS vs. Control) and # p<0.05 (Baicalein vs. LPS).

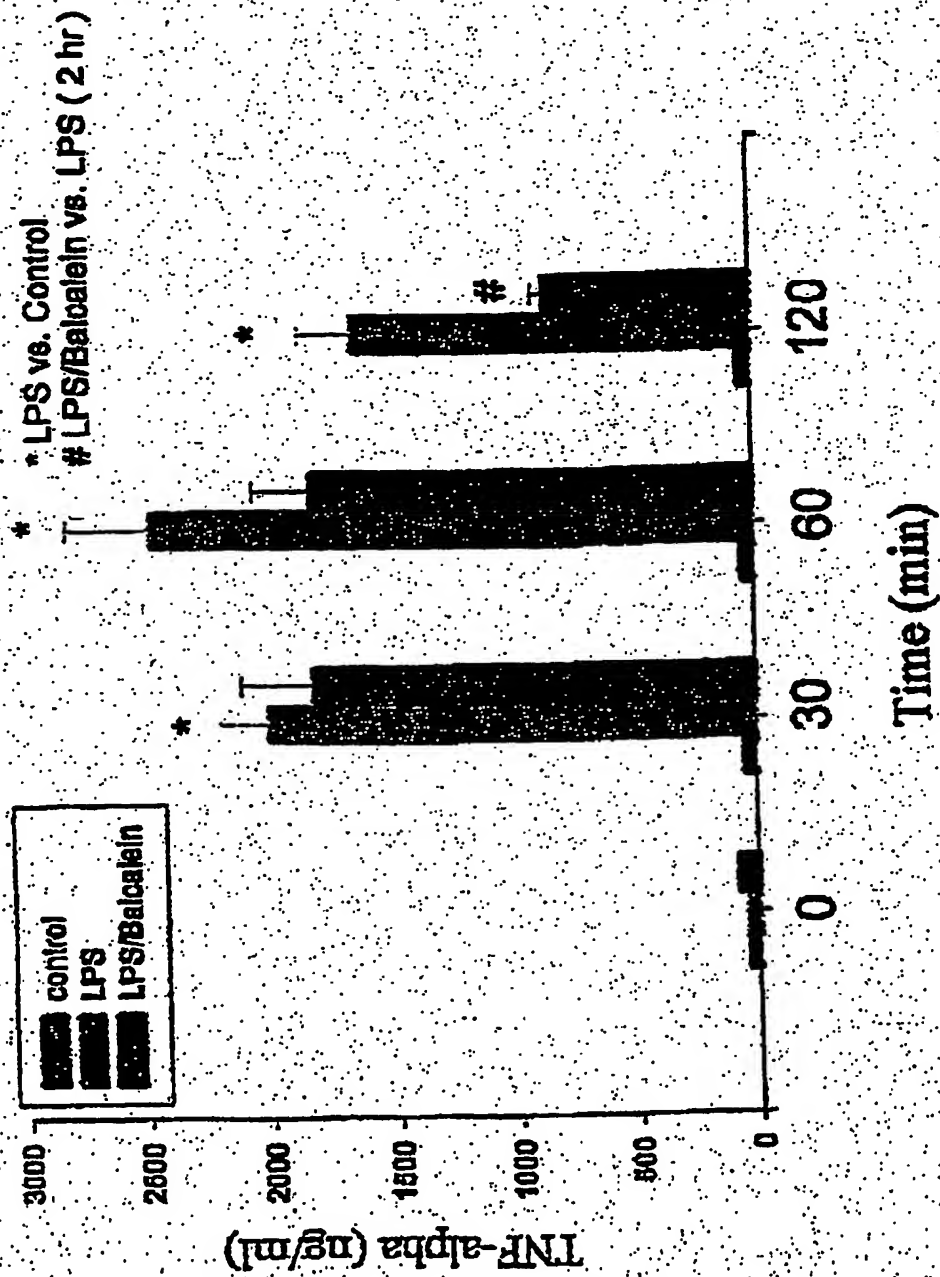


Figure 8 Effects of balcalein treatment on the plasma TNF- α level in LPS treated rats ($n=6$). Data represents as means \pm S.E.M., * $p<0.05$ (LPS vs. Control) and # $p<0.05$ (Balcalein vs. LPS).

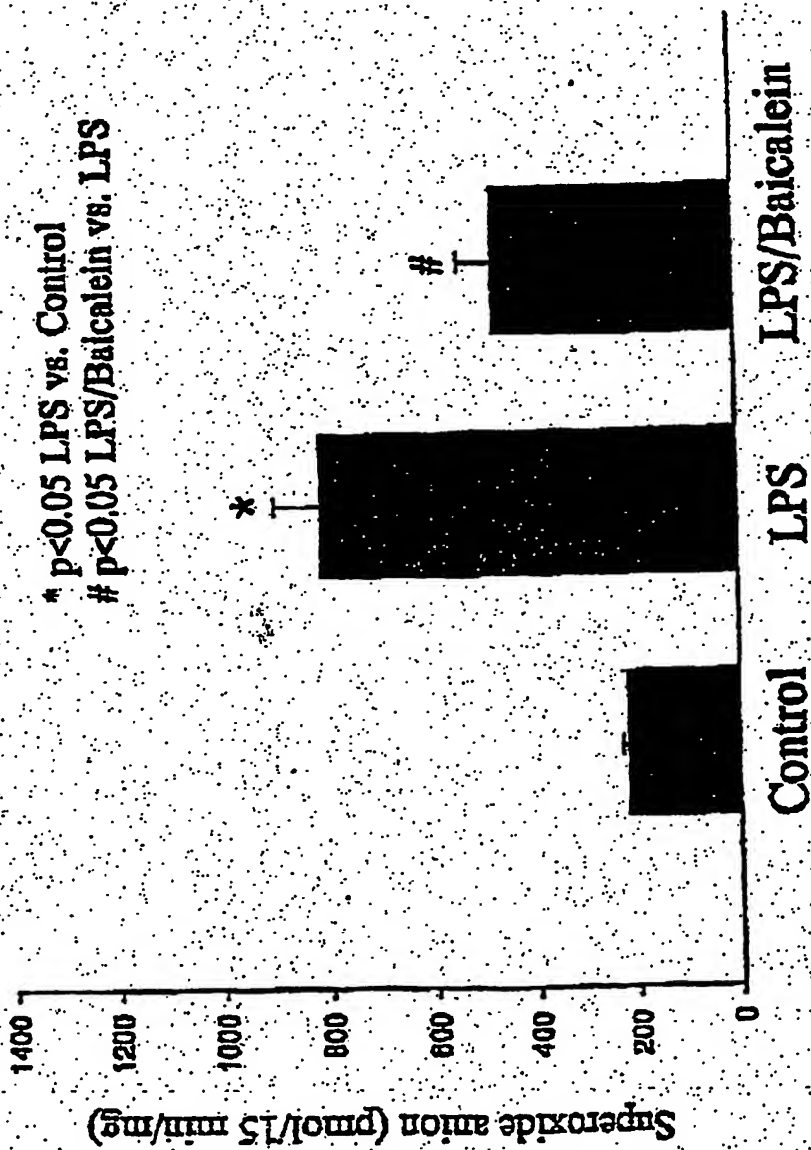


Figure 1. Effects of baicalein on superoxide anion in rats treated with endotoxin (n=8) (E. coli lipopolysaccharide, LPS). Data represents as means \pm S.E.M., * p<0.05 (LPS vs. Control) and # p<0.05 (Baicalein vs. LPS).

iNOS

β -actin

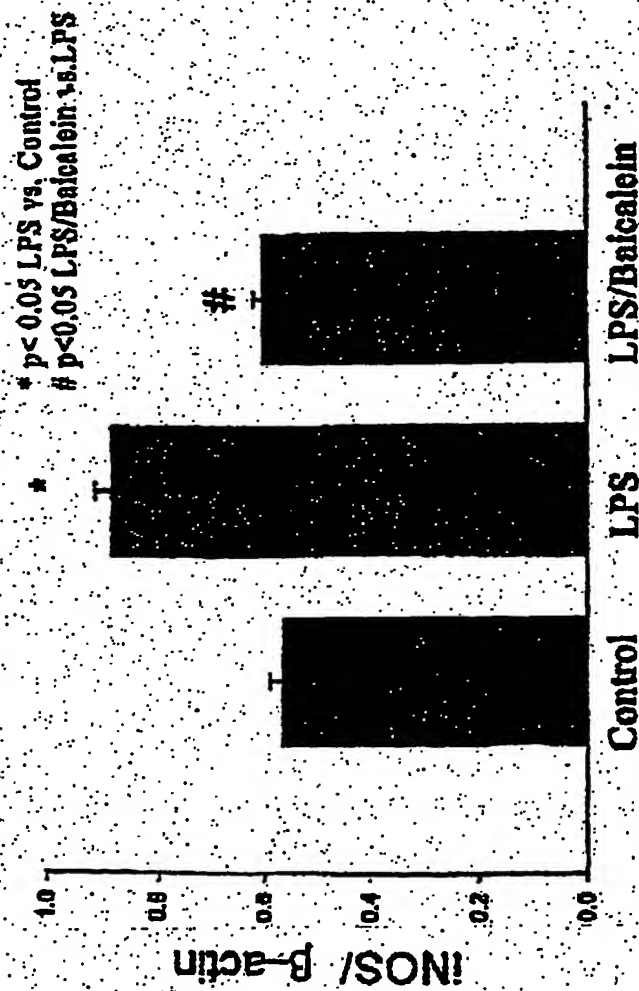


Figure 10. Effects of baicalein on iNOS protein expression in rats treated with endotoxin ($n=3$) (*E. coli* lipopolysaccharide, LPS). Data represents as means \pm S.E.M., * $p < 0.05$ (LPS vs. Control) and # $p < 0.05$ (Baicalein vs. LPS).

I κ B α

6 hr

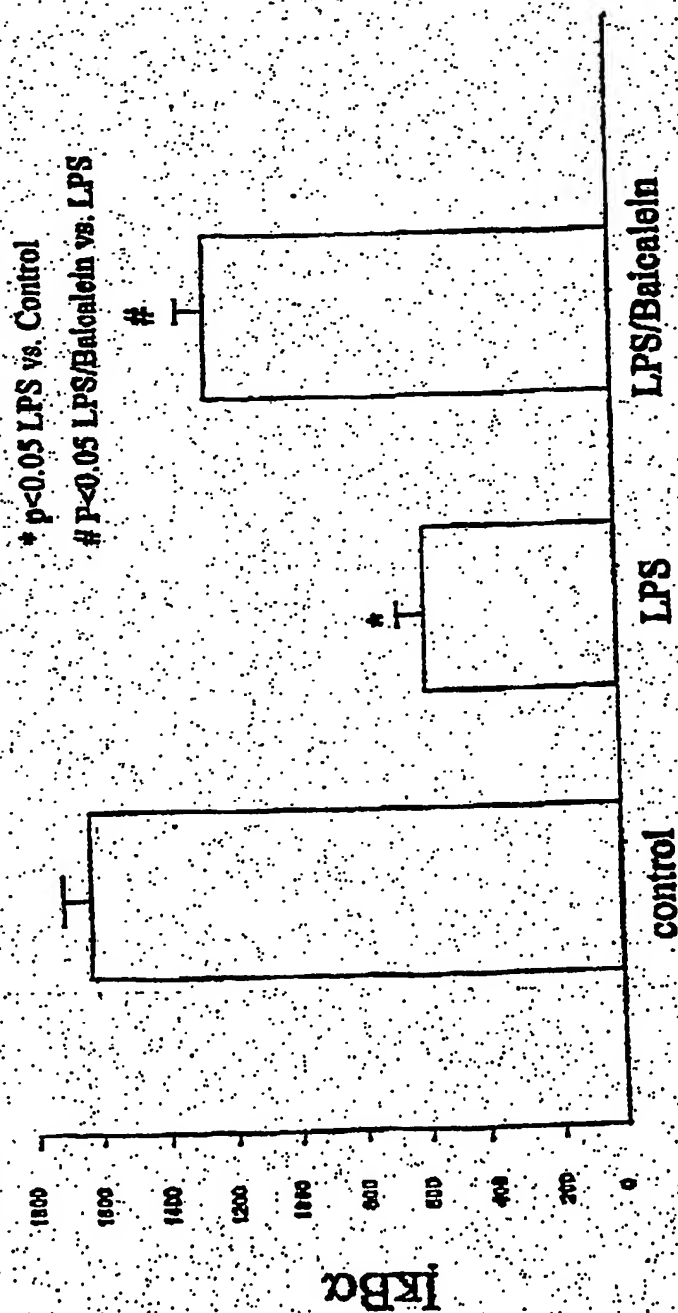


Figure 11. Effects of balcalein on I κ B- α protein degradation in rats treated with endotoxin at 6 hr (n=3) (E. coli lipopolysaccharide, LPS). Data represents as means \pm S.E.M., * p<0.05 (LPS vs. Control) and # p<0.05 (Balcalein vs. LPS).

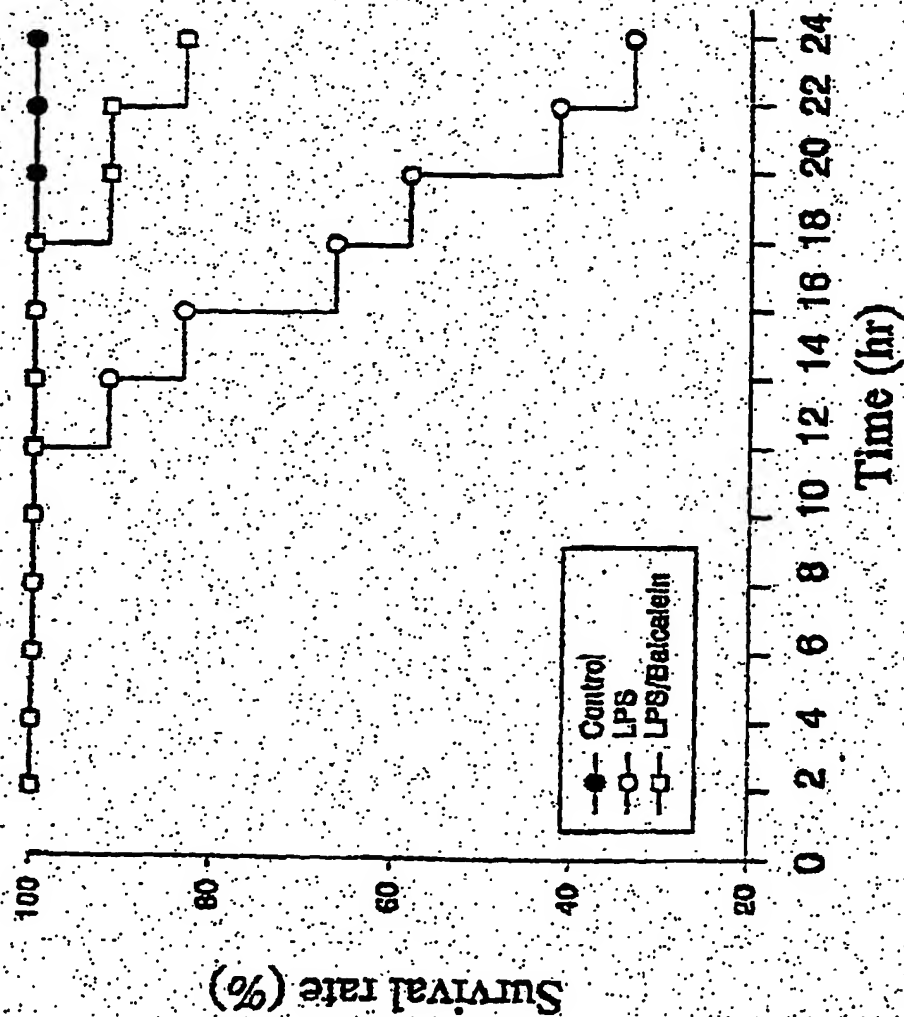


Figure 12. Effects of baicalcin on the survival rate treated with endotoxin (*E. coli* lipopolysaccharide, LPS, 50 mg/kg, i.p.) in mice (each groups, N=12) Data represents as means \pm S.E.M., * $p < 0.05$ (LPS vs. Control) and # $p < 0.05$ (Baicalcin vs. LPS).

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